

unexpected properties of the instant compounds. As noted by the Examiner, such an affidavit has not yet been presented. Such an affidavit is being presented with the instant amendment. The reason such an affidavit was not presented earlier is that in compiling the material for the affidavit, considerable data had to be assembled and audited for accuracy. Such a process took several months and final reports consisted of discussions and tables in excess of 1100 pages. In addition, these reports had to be examined by the Affiant and the comparison report prepared, all of which took additional time. The Examiner is respectfully requested to accept the instant affidavit at this time in support of the patentability of the instant application.

During the conference of the undersigned and his supervisor Dr. Westlake with the Examiner Mrs. Hazel and her Primary Examiner Mrs. Brown, on 17 July 1979 these matters were discussed. Applicants Attorney presented a draft of the attached affidavit to the Examiner. In addition, the complete reports of the toxicological activity of C-076 and of 22,23-dihydro C-076 Bla/ Blb were shown to the Examiner for purposes of discussion. It was indicated by Applicant that the complete reports, because of their bulk (totalling about 1100 pages) would not be made of record as it was felt that the affidavit, with its attachments, was sufficiently complete for the purpose of demonstrating the superior properties of the instant compounds. However, Applicants stated at the conference, and reiterate here, that if, at any time during the prosecution of this case, or thereafter, the Patent and Trademark Office wishes that the complete reports, or any portion thereof, be made of record, Applicants Attorney will promptly so provide. The complete reports will be maintained by Applicants in perpetuity for such purposes.

Thus it was agreed among the participants at the conference that the complete reports, because of their bulk, would not be made part of the affidavit. The most pertinent parts of the complete report will be made part of the affidavit, with the complete report

made available at any time at the discretion of the Examiner.

In addition, the reasons for the affidavit not being presented earlier (the review of the considerable amount of data necessitating a longer period of study than anticipated) were discussed and the Examiner agreed to allow the introduction of the affidavit.

The affidavit, which was discussed during the conference with the Examiner, and which is being presented herewith is the sworn statement of Dr. Richard Robertson. Dr. Robertson is a toxicologist employed by the Merck Institute for Therapeutic Research. The role of toxicologist is to study the effects of drugs upon the animal and human system, not with regard to their efficacy, or curative effects, but rather of the toxic or the deleterious effects of the drugs upon animal and human systems. As potential new drugs are developed by the Merck Sharp and Dohme Research Laboratories, the drugs are assayed for toxicity. This involves many tests upon several different species of animals, and uses a great number of animals for each test in order that a statistically significant result is obtained. When the potential new drug is at a stage of development where a New Drug Application or a New Animal Drug Application of the prepared, all of the toxicological tests are compiled, audited for accuracy and collected into a complete report. The auditing procedure is an involved one wherein the reports are compared with the original data. The comparison is made in some cases on each point of data in the original report, going back through any intermediate reports and to the originally recorded data. In other cases, where the data is extensive, samples are selected using selection procedures designed to insure that statistical accuracy will be maintained. When the complete reports are audited and released, they are presented to the U.S. Food and Drug Administration in support of the New Drug Application

or New Animal Drug Application.

Part of the function of Dr. Robertson is to oversee the compilation of certain of these reports. In the case of the compound of the instant invention and of the parent unsaturated compounds such reports were prepared under his supervision. Then, in order to properly assess the differences in toxicity between such compounds he prepared a comparison report in which the toxicities of the two types of compounds were discussed and compared. This report is unincorporated in its entirety into the affidavit. The Exhibits in the affidavit consist of the summaries and tables of contents of the two Complete Reports.

After the introduction, in which the general structures of the compounds and the various tests which are being compared, are discussed, the section "Comparison of Results" is found. The report states that in the Ames bacterial mutagen test, neither the unsaturated nor the dihydro compound was mutagenic.

The next paragraph discusses the LD50 values of the compounds. This is defined as the "calculated single dose that will kill 50 percent of the test animals". At equivalent dosing concentrations (0.8%) the C-076 Bla compound has an LD50 one half to one third that of the dihydro compound. This data is found on page 1 of each of Exhibits I and III. The only data point for C-076 Bla (Exhibit III) which is comparable with the dihydro compound in the female CF1 mouse at 0.8% concentration. The 0.2 concentration is not directly comparable since toxicity can vary with dose concentration. This LD50 value is 13.6 mg. Exhibit I lines 1 and 2 of the table on page 1 gives the LD 50 for the dihydro Bla compound (L-638,-709). This is given as 87.2 and 31.7 mg/kg. the next two lines are the LD50 for the dihydro Blb compound (L-638,622) and are given as 56.6 and 27.6. The 6th to the 9th lines are for mixture of Bla and Blb (approximately 80:20) and are given as 24.6, 27.1, 41.6 and 40.0 mg/kg. These values are all seen to be much greater than the 13.6 mg/kg for C-076 Bla. (The 10th to 14th lines are not directly comparable to the C-076 Bla

results because of different animals, sexes of animals and the like). These figures support the statement in the comparison report that the C-076 B1a compound has one third to one half the LD50 toxicity than the dihydro compound for equivalent concentration.

However, taking the C-076 B1a rat tests at 0.2% solution and comparing it with the 0.8% solution for the 22,23-dihydro compounds, it is noted that the latter is 4 to 5 times less toxic (lines 6 to 12 in the last paragraph of page 3 of the affidavit). Also, owing to variation in LD50 due to concentration variances, the difference may be even greater.

The last line on the table on page 1 of Exhibit III and the fifth line of the table on page 1 of Exhibit I list the LD50 values for neonatal (newborn) rats for C-076 B1a and the dihydro compound respectively. They are listed as 1.52 for C-076 B1a and 2.3 for the dihydro compound. Thus, even in this sensitive test the C-076 B1a compound had a 35% smaller LD50 than the dihydro compound.

The next paragraph of the Summary Report discusses the oral reproduction studies. This test consists of two parts. The first part is the administration of the drug to pregnant female rats on a daily basis from before mating through lactation. The second part consists of administration of the drug to the offspring of these pregnant rats for 90 days.

In the in utero study MK933 was administered at levels of 0.4, 0.8 and 1.6 mg/kg. The no-effect level is determined by observing the offspring. For MK933 the no-effect level was 0.4 mg/kg (pg. 4, second paragraph, line 2). The only effect observed at higher levels was "some intravascular hemolysis" (separation of hemoglobin from red blood cells and its appearance in plasma occurring in the vascular system) which was only observed at the completion of the 90 day test. At 1.6 mg/kg there was "a very slight increase in mortality and some hypothermia".

Contrasting with this, the C-076 B1a had no-effect level of 0.1 mg/kg (pg. 4, paragraph 1, line 8) four times the level of

MK933. In addition, in comparison with the latter slight toxic signs at the higher doses of MK933, the C-076 B1a had, at 0.2 mg/kg the more severe toxic signs of "spastic movements and tremors". The toxicity at 0.5, 1.0 and 1.5 mg/kg in the in utero portion of the test was so great that these doses had to be discontinued and only the lower doses carried to the 90 day test.

Thus, in the in utero portion of the test, the dihydro compound is at least 4 times safer than the C-076 compound, and looking at the mild toxic signs of the dihydro compound compared with the severe toxic signs of the parent compound, the level is probably higher.

In the 90 day test neither the dihydro compound at 0.4, or 0.8 mg/kg/day, nor the parent compound at 0.1, 0.2 or 0.4 mg/kg/day shows any toxic symptoms. At 1.6 mg/kg/day the dihydro compound did show some slight spleen enlargement.

The oral reproduction study in rats for the dihydro compound is discussed in the Summary in Exhibit I, Section 7 starting on page S-6. The same study for the parent C-076 compound is discussed in the Summary in Exhibit III, Sections 6 and 7, starting on page S-6. (It is noted that the higher doses were tested in the test described in Section 6 and the lower doses in Section 7.) The 90 day test for the offspring of the in utero study for the dihydro compound is discussed in Exhibit I, Section G, starting on page S-8. The same test for the C-076 compound is discussed in Exhibit III Section 9, starting on page S-13.

The next test discussed in the comparison report is the 18 week oral toxicity study in dogs. C-076 B1a was tested at 0.25, 0.5, 2.0 and 8.0 mg/kg/day. The no-effect level was 0.25 mg/kg/day. It is noted that "Death, tremors ataxia (failure of muscular coordination; irregularity of muscular action) and mydriosis (extreme or morbid dilation of the pupil) was observed at all other doses" MK933 was administered at 0.5, 1.0 and 2.0 mg/kg/day and had a no-effect level of 0.5 mg/kg/day (twice the level

of the parent compound.) Also the dihydro compound only exhibited the above symptoms at the highest level tested (2.0 mg/kg). No dogs could survive the test with the parent compound at 2.0 mg/kg/day or higher. One half the dogs could survive 94 to 95 consecutive 2.0 mg/kg doses.

These dogs trials are discussed in Exhibit I, Section H, starting on page S-9 for the dihydro compound and in Exhibit III, Section 8, starting on page S-10 for the parent compound.

The last test discussed in the comparison report is the mouse teratology (production of physical defects in utero) study. This study again is in two parts; one being the observation of birth defects in the offspring (teratogenicity), and observation of signs of toxicity to the pregnant mouse (maternotoxicity).

C-076 Bla was tested in the pregnant mouse at 0.2, 0.4 and 0.8 mg/kg/day and found to be maternotoxic at all levels with a teratogenic no-effect level of 0.2 mg/kg/day. The test was then run again at lower levels to determine the no-effect level for maternotoxicty which was found to be 0.05 mg/kg/day. The teratogenic effects noticed were cleft palates.

The same tests were carried out on the individual components of MK933 at levels of 0.2, 0.4, 0.8 and 1.6 mg/kg/day for the dihydro C-076 Bla and at 0.4, 0.8 and 1.6 mg/kg/day for the dihydro C-076 Blb component. The teratogenic no-effect levels obtained were 0.8 mg/kg/day for dihydro C-076 Bla and 0.4 mg/kg/day for dihydro C-076 Blb. (Compared with 0.2 mg/kg/day for the parent C-076 Bla) The maternotoxicty no-effect levels obtained were 0.2 mg/kg/day for dihydro C-076 Bla and 0.4 mg/kg/day for dihydro C-076 Blb. (Compared with 0.05 mg/kg/day for the C-076 Bla)

The teratogenic results are summarized in lines 17-23 of the first full paragraph on page 5 of the affidavit when it is stated:

"Based on maternal mortality in these mouse teratology studies, C-076 (Bla) is approximately four and eight times more toxic than the 22,23 dihydro C-076(Bla) and 22,23 dihydro C-076 (Blb) components of MK933

respectively. C-076 (B1a) also had approximately four and two times the teratogenic potential in mice of the 22,23 dihydro C-076 (B1a) and 22,23 dihydro C-076 (B1b) components of MK933, respectively." (emphasis added)

The mouse teratology studies for the dihydro compounds are discussed in Exhibit I, Section C,D and E, starting on page S-3. The same studies for the parent compound are discussed in Exhibit III Section 3,4 and 5, starting on page S-3.

Thus, it is readily seen that the hydrogenation of the 22,23 double bond in the parent C-076 series results in compounds with a considerable degree of safety advantage. The following table summarizes the various toxicological tests discussed and gives the multiples of safety advantage achieved by the 22,23-dihydro compounds:

<u>Test</u>	<u>Multiples of Safety Advantage For Dihydro Compounds</u>
LD50 (mouse)	2 to 3 times
LD50 (rat-different concentration)	4 to 5 times
LD50 (infant rat)	35% increase
Oral reproduction (neonatal rat)	8 times
Oral reproduction (rat-irrespective of age)	4 times
18 week oral toxicity (dog)	2 times
Mouse teratology (dihydro C-076 B1a)	4 times
Mouse teratology (dihydro C-076 B1b)	8 times
Mouse maternotoxicity (dihydro C-076 B1a)	4 times
Mouse maternotoxicity (dihydro C-076 B1b)	2 times

It is respectfully submitted that the toxicity data discussed above and attached hereto demonstrates, without question, that the saturation of the double bond at the 22,23 position results in an unexpected and unpredictable decrease in toxicity. This data convincingly removes the instant claims from any presumption of obviousness which could be attributed to the parent unsaturated compounds. Such parent compounds have 5 unsaturations. The instant application has selectively reduced one of them. The remaining four unsaturations

are untouched. With this change there results a modification of the toxicological properties which is completely out of proportion to the size of the chemical change. It is respectfully submitted that the instant claims are unobvious modifications over the prior art and the Examiner is respectfully requested to reconsider and withdraw the rejection.

Claims 15 and 16 stand rejected as being obvious over the Kishi and Chemical Abstracts references. Claims 15 and 16 having been cancelled, the rejection is completely avoided.

All of the rejections of the official Action of April 20, 1979 having been attended to with the foregoing remarks and the attached affidavit, Applicants Attorney respectfully submits that the instant claims are in condition for allowance. A prompt notice of Allowance is respectfully solicited.

Respectfully submitted

By



David L. Rose Reg. No. 26,332
P.O. Box 2000
Merck and Co., Inc.
Rahway, NJ 07065

20 July 1979